

Listing of the Claims

This listing of claims will replace all prior versions and listings of claims in the application:

1. (Currently amended) A method of assessing an amount of a known target nucleic acid sequence in a sample comprising:
 - a) co-amplifying using a duplex PCR said known target nucleic acid sequence and a known amount of a known control nucleic acid sequence using different target and control specific primers to produce respective target and control amplicons, wherein said control nucleic acid sequence is different than said target nucleic acid sequence, and wherein said co-amplifying is stopped during an exponential phase of the duplex PCR; [[and]]
 - b) predetermining a dispensation order of nucleotides based on the nucleotide sequence order of the target nucleic acid and control nucleic acid sequence;
 - c) dispensing the nucleotides in a single reaction in the predetermined dispensation order in a primer extension reaction such that the target and control nucleic acids are extended sequentially; and
 - [[b)]] d) determining relative amounts of said target and control respective amplicons ~~by using an extension primer and extending the extension primer sequentially in a primer extension reaction using a sequential dispensation of nucleotides in a predetermined order such that the target and the control amplicons are extended sequentially to allow analysis of each amplicon separately in a single reaction mixture thereby allowing determination of the relative quantity of the target nucleic acid sequence compared to the control nucleic acid sequence,~~
~~wherein determining the relative quantity of the target nucleic acid sequence compared to the control nucleic acid sequence comprises~~ comparing a quantity of nucleotides incorporated during said primer extension reaction for said target amplicon with a quantity of nucleotides incorporated during said primer extension reaction for said control amplicon, wherein relative amounts of said respective amplicons are proportional to relative quantities of nucleotides incorporated during said primer extension reactions

- and said amount of said target nucleic acid sequence in said sample is proportional thereto.
2. (Original) The method of claim 1, wherein said control nucleic acid is an endogenous or exogenous nucleic acid.
 3. (Original) The method of claim 1, wherein said primer extension reaction is performed using identical primers for said respective target and control amplicons.
 4. (Original) The method of claim 1, wherein said primer extension reaction is performed using a different template-specific primer for said respective target and control amplicons.
 5. (Previously presented) The method of claim 4, wherein said primer extension reaction is detected by detecting pyrophosphate (PPi) release.
 6. (Original) The method of claim 5, wherein said pyrophosphate is detected luminometrically.
 7. (Original) The method of claim 6, wherein said pyrophosphate is detected enzymatically using the enzyme luciferase as a PPi-detection enzyme.
 8. (Original) The method of claim 7, wherein in the primer extension reaction, an a-thio analogue of an adenine nucleotide is used.
 9. (Previously presented) The method of claim 8, wherein said target nucleic acid and control nucleic acid are co-amplified using amplification primers which are immobilized or carry means for immobilization.
 10. (Previously presented) The method of claim 9, wherein more than one different control nucleic acid is used.
 11. (Original) The method of claim 10, wherein different amounts of each different control nucleic acid are used.
 12. (Previously presented) The method of claim 11, wherein said primer extension reaction is performed on said respective target and control amplicons using identical primers for said respective target and control amplicons.
 13. (Previously presented) The method of claim 11, wherein said primer extension reaction is performed on said respective target and control amplicons using a different template-specific extension primer on each said amplicon.

14. (Previously presented) The method of claim 13, wherein multiple primer extension reactions are performed on each of said respective target or control amplicons.
15. (Original) The method of claim 14, wherein each said primer extension reaction yields an extension product of different length or sequence.
16. (Previously presented) The method of claim 15, wherein said target nucleic acid is selected from the group consisting of a gene or a fragment thereof, a T- DNA region or a fragment thereof, a vector or a fragment thereof, a plasmid or fragment thereof, a nucleic acid polymorphism and a non-coding region of a nucleic acid sequence.
17. (Previously presented) The method of claim 16, wherein said gene or fragment thereof is a gene selected from the group consisting of a disease resistance gene, an antibiotic resistance gene and a gene conferring an investigated trait.
18. (Currently amended) A method of assessing copy number of a target nucleic acid sequence in a genome of an organism comprising:
 - a) co-amplifying using a duplex PCR said target nucleic acid sequence and a known amount of a known control nucleic acid sequence using different target and control specific primers to produce respective target and control amplicons, wherein said control nucleic acid sequence is different than said target nucleic acid sequence, wherein said co-amplifying is stopped during an exponential phase of the duplex PCR; [[and]]
 - b) predetermining a dispensation order of nucleotides based on the nucleotide sequence order of the target nucleic acid and control nucleic acid sequence;
 - c) dispensing the nucleotides in a single reaction in the predetermined dispensation order in a primer extension reaction such that the target and control nucleic acids are extended sequentially; and
 - [[b)]] d) determining relative amounts of said target and control ~~respective amplicons by using an extension primer and extending the extension primer sequentially in a primer extension reaction using a sequential dispensation of nucleotides in a predetermined order such that the target and the control amplicons are extended sequentially to allow analysis of each amplicon separately in a single reaction mixture thereby allowing determination of the relative~~

~~quantity of the target nucleic acid sequence compared to the control nucleic acid sequence,~~

~~wherein determining the relative quantity of the target nucleic acid sequence compared to the control nucleic acid sequence comprises~~ comparing a quantity of nucleotides incorporated during said primer extension reaction for said target amplicon with a quantity of nucleotides incorporated during said primer extension reaction for said control amplicon, wherein relative amounts of said respective amplicons are proportional to relative quantities of nucleotides incorporated during said primer extension reactions and copy number of said target nucleic acid sequence in said genome is proportional thereto.

19. (Original) The method of claim 18, wherein said control nucleic acid is an endogenous or exogenous nucleic acid.
20. (Previously presented) The method of claim 19, wherein said target nucleic acid is selected from the group consisting of a gene or a fragment thereof, a T-DNA region or a fragment thereof, a vector or a fragment thereof, a plasmid or fragment thereof, a nucleic acid polymorphism and a non-coding region of a nucleic acid sequence.
21. (Previously presented) The method of claim 20, wherein said gene or fragment thereof is a gene selected from the group consisting of a disease resistance gene, an antibiotic resistance gene, and a gene conferring an investigated trait.
22. (Currently amended) A method of assessing copy number of a plasmid in a cell comprising:
 - a) co-amplifying using a duplex PCR a target nucleic acid sequence from said plasmid and a known amount of a known control nucleic acid sequence using different target and control specific primers to produce respective target and control amplicons, wherein said control nucleic acid sequence is different than said target nucleic acid sequence, wherein said co-amplifying during an exponential phase of the duplex PCR; [[and]]
 - b) predetermining a dispensation order of nucleotides based on the nucleotide sequence order of the target nucleic acid and control nucleic acid sequence;

c) dispensing the nucleotides in a single reaction in the predetermined dispensation order in a primer extension reaction such that the target and control nucleic acids are extended sequentially; and

[[b)] d) determining relative amounts of said target and control respective amplicons ~~by using an extension primer and extending the extension primer sequentially in a primer extension reaction using a sequential dispensation of nucleotides in a predetermined order such that the target and the control amplicons are extended sequentially to allow analysis of each amplicon separately in a single reaction mixture thereby allowing determination of the relative quantity of the target nucleic acid sequence compared to the control nucleic acid sequence;~~

~~wherein determining the relative quantity of the target nucleic acid sequence compared to the control nucleic acid sequence comprises~~ comparing a quantity of nucleotides incorporated during said primer extension reaction for said target amplicon with a quantity of nucleotides incorporated during said primer extension reaction for said control amplicon, wherein the relative amounts of said respective amplicons are proportional to the relative quantities of nucleotides incorporated during said primer extension reactions and said copy number of said plasmid in said cell is proportional thereto.

23. (Original) The method of claim 21, wherein said control nucleic acid is an endogenous or exogenous nucleic acid.

24. (Currently amended) A method of identifying an organism having a single copy of a target nucleic acid sequence comprising:

a) co-amplifying using a duplex PCR said target nucleic acid sequence and a known amount of a known control nucleic acid sequence using different target and control specific primers to produce respective target and control amplicons, wherein said control nucleic acid sequence is different than said target nucleic acid sequence, wherein said co-amplifying is stopped during an exponential phase of the duplex PCR;

b) predetermining a dispensation order of nucleotides based on the nucleotide sequence order of the target nucleic acid and control nucleic acid sequence;

c) dispensing the nucleotides in a single reaction in the predetermined dispensation order in a primer extension reaction such that the target and control nucleic acids are extended sequentially;

[[b)]] d) determining relative amounts of said target and control respective amplicons ~~by using an extension primer and extending the extension primer sequentially in a primer extension reaction using a sequential dispensation of nucleotides in a predetermined order such that the target and the control amplicons are extended sequentially to allow analysis of each amplicon separately in a single reaction mixture thereby allowing determination of the relative quantity of the target nucleic acid sequence compared to the control nucleic acid sequence;~~

~~wherein determining the relative quantity of the target nucleic acid sequence compared to the control nucleic acid sequence comprises comparing a quantity of nucleotides incorporated during said primer extension reaction for said target amplicon with a quantity of nucleotides incorporated during said primer extension reaction for said control amplicon, wherein relative amounts of said respective amplicons are proportional to relative quantities of nucleotides incorporated during said primer extension reactions and said copy number of said target nucleic acid sequence is proportional thereto, and~~

e) ~~determining if said copy number of said target nucleic acid sequence is one,~~
wherein if a copy number of said target nucleic acid sequence is equal to one it is indicative of an organism having a single copy of said target nucleic acid sequence.

25. (Original) The method of claim 24, wherein said control nucleic acid is an endogenous or exogenous nucleic acid.
26. (Previously presented) The method of claim 25, wherein said target nucleic acid is selected from the group consisting of a gene or a fragment thereof, a T-DNA region or a fragment thereof, a vector or a fragment thereof, a plasmid or fragment thereof, a nucleic acid polymorphism and a non-coding region of a nucleic acid sequence.
27. (Previously presented) The method of claim 26, wherein said gene or fragment thereof is a gene selected from the group consisting of a disease resistance gene, an antibiotic resistance gene, and a gene conferring an investigated trait.

28. (Previously presented) The method of claim 27, wherein said organism is a genetically modified organism.
29. (Original) The method of claim 28, wherein said organism is identified from a plurality of organisms.
30. (Previously presented) The method of claim 29, wherein said organism is an organism selected from the group consisting of mammals, plants, birds, bacteria, fungi, and viruses.
31. (Previously presented) The method of claim 1, wherein said control nucleic acid is acetohydroxyacid synthase (AHAS).
- 32.-34. (Cancelled)
35. (Previously presented) The method of claim 1, wherein said target nucleic acid is a chromosome or a fragment thereof and assessing an amount of said target nucleic acid is used to determine chromosome copy number.
36. (Previously presented) The method of claim 1, wherein said target nucleic acid is selected from the group consisting of neomycin phosphotransferase (NPTII) phosphinothricin acetyl transferase (PAT), hygromycin phosphotransferase (HPT), acetolactate synthase (ALS, also referred to as acetohydroxyacid synthase (AHAS)), phosphomannose isomerase (PMI), 5-enolpyruvylshikamate-3-phosphate synthase (EPSP synthase), oxalate oxidase, adenosine deaminase, dihydrofolate reductase, thymidine kinase, xanthine-guanine phosphoribosyltransferase, and blasticidin S deaminase.
37. (Cancelled)
38. (Previously presented) The method of claim 1, wherein said control nucleic acid sequence is selected from the group consisting of acetolactate synthase, actin, profilin, ferredoxin-dependent glutamate synthase, ribose-phosphate pyrophosphokinase, beta-ketoacyl-CoA synthase, delta-5 fatty acid desaturase, sequence-tag sites (STS) of known copy number and non-translated sequences of known copy number.
39. (Previously presented) The method of claim 1, wherein said primer extension reaction is performed using a primer specific for NPTII and another primer specific for AHAS.